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Material for Demonstrating Sexuality in the Ascomycetes

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Nothing since the time of Pasteur and Koch has done more to revolutionize our ideas regarding the nature of fungi than the art of culturing them from single spores. When our corn, wheat, cotton and fruit crops aggregate several billions of dollars in value annually and we realize that pathogenic fungi frequently take toll of a large percentage, it would seem that the student in Botany and Biology should be privileged at least a nodding acquaintance with a representative of each of the large groups of fungi.

The question is, what is the best form in each case for class-room demonstration. Any species, to fulfil these requirements should first of all be readily available. It must be one that will go through its life cycle in culture. It should show both types of reproduction, asexual and sexual, and produce fruiting bodies readily demonstrable, at least under the low power of the microscope. Other things being equal, a form which is heterothallic or haplo-dioecious* will prove far more interesting to students. For example, among the Phycomycetes, *Rhizopus nigricans* and *Phycomyces Blakesleeanus* serve admirably for the purpose. Any student who inoculates a petri dish culture with the two strains of the latter species and sees for the first time the row of zygospores developing along the line where the two mycelia meet,

* In dioecious flowering plants the stamens and pistils are borne on different individuals. A heterothallic or haplodioecious fungus is merely analagous to a dioecious flowering plant. It is one in which an individual haploid gametophytic mycelium is either of one sex or the other. Sexual reproduction occurs only when two mycelia of opposite sex are mated or grown together. A homothallic or haplomonoecious fungus is one in which each individual mycelium is capable of reproducing sexually and if differentiated sex organs are developed both kinds will be formed on the same mycelium.

and contrasts this picture with one obtained by inoculating a culture on opposite sides with two doses of the same strain, is fully impressed with the necessity for mating two strains of opposite sex in order to lead to sexual reproduction.

No ascomycete so far discovered serves better to demonstrate sexual reproduction than Pyronema. The beautiful rose colored ascocarps are frequently seen in places where brush piles have been burned over a few weeks previously. It fruits abundantly in petri dish cultures on many kinds of agar media, and

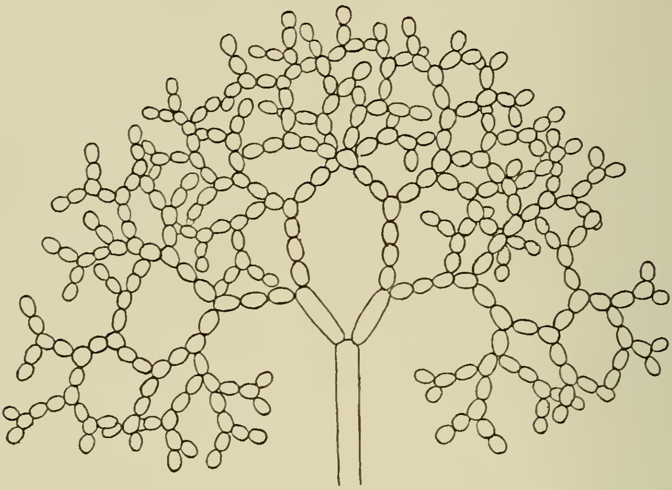

Figure 1.

Monilioid conidia, asexual spores, of *Neurospora sitophila*. Sporophores branch dichotomously and especially when young show a beautiful symmetry

the ascospores remain viable for a long time. Numerous oogonia and antheridia which can be readily distinguished are developed in the cultures. One objection to Pyronema is that it does not produce asexual spores and the ascocarps do not show simple striking distinguishing features. Furthermore, the species is haplo-monoecious. *Ascobolus magnificus*, a species whose ascocarps present a very striking appearance, is heterothallic and develops very large oogonia and antheridia. The ascospores are beautifully colored. It is a very rare species, however, and one not easily cultured. *Ascobolus carbonarius* is rather common on burned places and develops large sex primordia, but this species also is not readily cultured.

The ideal ascomycetes for classroom work are the species of the orange-colored monilioid molds in which the conidia or asexual spores are borne in chains (Fig. 1) and which have as their perfect or sexual stage the pyrenomycete Neurospora (Fig. 2). The ascocarp or perithecium has a well differentiated wall and ostiole, apical opening, and is small enough to be

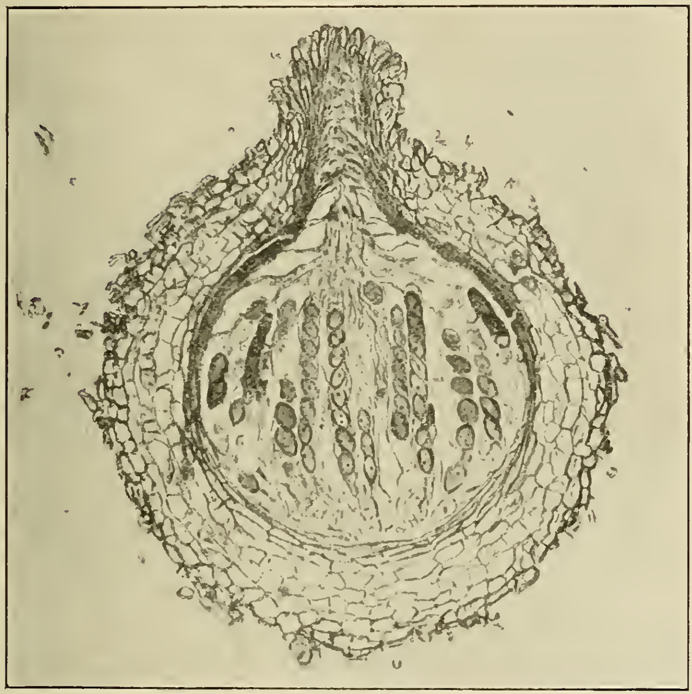

Figure 2. Ascocarp or perithecium of *Neurospora sitophila*, ×200.

examined under the microscope. The asci are few in number and develop large colored spores with ribbed markings. (Plate 2). These fungi reproduce themselves asexually by means of spores formed on dichotomously branched conidiophores. *Neurospora sitophila* is the common orange-colored mold of the bakery. *N. crassa* grows everywhere in the tropics on sugar cane bagasse and on burned over places in forests. Both are heterothallic or haplodioecious. *N. tetrasperma* is known only from a few places as yet, but would serve the best for classroom work, because it is either homothallic or heterothallic, depending upon the strains being cultured. It does not produce very many of the monilioid asexual spores. This is an advantage, because the details of the development of the asococarps can be more readily studied on this account. Normally the mycelium is homothallic, or haplomonoecious. One can always obtain heterothallic strains by selecting small ascospores. In this case asocarps will be produced only when two mycelia of opposite sex are mated in

Figure 3. Petri dish culture inoculated with two strains of opposite sex of *Neurospora tetrasperma* showing characteristic distribution of ascocarps. The black smudged areas show where the ascospores have been discharged.

culture. Figure 3 shows a petri dish culture in which the unisexual strains S_1 and S_6 have been growing for several days on corn meal agar. Fruit bodies are first formed along the line where the two mycelia meet, after which they appear one by one along the lines of radiately growing hyphae of the strain S_6. Just why the stimulus of fertilization results in a production of ascocarps back along the lines of mycelial growth is not yet known. However, the fruit bodies from this combination always form on the mycelium of the strain S_6, so that it looks as though the sexes are differentiated and that strain S_6 develops the